42108/26146

METHODS AND COMPOSITIONS FOR CONTROLLING PROTEIN ASSEMBLY OR AGGREGATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation-in-part of U.S. Patent Application Serial No. 09/677,500, filed October 2, 2000, which is a continuation-in-part of U.S. Patent Application Serial No. 09/657,554, filed September 8, 2000, a continuation-in-part of U.S. Patent Application Serial No. 09/657,989, filed September 8, 2000, and a continuation-in-part of U.S. Patent Application Serial No. 09/127,620, filed September 8, 2000, which is now U.S. Patent No. 6,127,393, which is a continuation-in-part of U.S. Patent Application Serial No. 08/843,157, filed April 11, 1997, now abandoned, which is a continuation-in-part of U.S. Patent Application Serial No. 08/581,351, filed December 29, 1995, now U.S. Patent No. 5,767,135, which claims priority to U.S. Provisional Patent Application Serial No. 60/024,221, filed October 22, 1996 and to U.S. Provisional Patent Application Serial No. 60/026,992, filed September 20, 1996.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not Applicable.

REFERENCE TO A MICROFICHE APPENDIX

Not Applicable.

FIELD OF THE INVENTION

This invention relates to compositions and methods for the treatment of degenerative diseases. More specifically, the invention relates to pharmaceutical compositions and methods for the treatment of degenerative diseases related to aggregation or assembly of conformationally altered proteins including, but not limited to Alzheimer's disease, cerebral amyloid angiopathy, Parkinson's disease, frontal temporal dementia, Pick's disease, amyotrophic lateral sclerosis, Huntington's disease, bovine spongiform encephalopathy and Creutzfelds-Jakob disease.

BACKGROUND OF THE INVENTION

Assembly or aggregation of conformationally altered proteins is thought to be a major cause of prepathological and pathological conditions including amyloidoses, prion diseases, and other common degenerative diseases. Conformational alterations from α-helical or random coil to β-sheet conformation are believed to be required for the conversion of normally soluble and functional proteins into insoluble and pathogenic states. Examples of such insoluble proteins include: Beta-Amyloid Precursor Protein (APP) and Beta-Amyloid (βA) in amyloid plaques of Alzheimer's Disease (AD), Familial AD (FAD) and cerebral amyloid angiopathy (CAA); α-synuclein deposits in Lewy bodies of Parkinson's disease; Tau in neurofibrillary tangles in frontal temporal dementia and Pick's disease; Superoxide Dismutase in amyotrophic lateral sclerosis; Huntingtin in Huntington's disease; and Prion Protein (PrP) in Creutzfelds-Jakob disease (CJD). These conformationally altered insoluble proteins are composed mostly of fibrils formed by the assembly or aggregation of β-sheet monomers. It is believed that abnormal binding of a metal ligand in the metal-binding sites of the normal, soluble proteins is a major factor in the pathogenesis and continued pathology of the resulting diseases. It has also been suggested that certain forms of these diseases may be inherited. However, no methods currently exist to definitively link these diseases to genetic inheritance.

Currently, there is no effective therapy for PrP infection. There are also no treatments currently available that target the conformational changes from α-helical or random coil to β-sheet conformation to treat cerebral amyloid angiopathy, Parkinson's disease, frontal temporal dementia, Pick's disease, amyotrophic lateral sclerosis, or Huntington's disease. AD therapeutic agents such as acetylcholinesterase (AChE) inhibitors that enhance cholinergic neurotransmission by hindering the breakdown of acetylcholine have been approved by the FDA. This approach, however, does not retard the progression of the underlying neurodegenerative disease.

Nonspecific chelation therapy has become increasingly promoted as a therapy for AD and other diseases manifested by the aggregation or assembly of conformationally altered proteins. However, the growing practice of intravenous infusions of well-known nonspecific chelators such as ethylene-diamine-tetraacetic acid (EDTA) can lead to systemic metal ion depletion, making its use less desirable.

Therefore, novel compositions and methods are needed to treat diseases caused by the prepathological and pathological assembly or aggregation of proteins causing amyloidoses, prion diseases and other degenerative diseases that inhibit or reverse the progression of the underlying neurodegenerative disease and do not result in metal ion depletion.

BRIEF SUMMARY OF THE INVENTION

In overcoming the above disadvantages, it is an object of the invention to produce compositions that may be used to successfully treat degenerative diseases.

Accordingly, and in one aspect of the invention, a composition capable of solubilizing a conformationally altered protein that includes a carboxylic acid anion of picolinic acid, analogs, or derivatives, thereof and a cation is provided, wherein the composition is not zinc picolinate, chromium picolinate, molybdenum picolinic, iron picolinic, manganese picolinate, copper picolinate, boron picolinate or vanadium picolinate.

In a second aspect of the invention, the above-described composition of the invention comprises picolinic acid, its analogs, or derivatives.

In a third aspect of the invention, the above-described composition of the invention comprises fusaric acid.

In a fourth aspect of the invention, a method of preventing or reversing conformationally altered protein assembly or aggregation in an animal is provided that includes introducing picolinic acid, its analogs, or derivatives to the conformationally altered protein.

In a fifth aspect of the invention, a method of preventing or reversing conformationally altered protein assembly or aggregation in an animal is provided that includes introducing fusaric acid to the conformationally altered protein.

In a sixth aspect of the invention, a method of treating conformationally altered protein assembly or aggregation in an animal is provided that includes administering a therapeutically effective amount of the above-described compositions of the invention.

These and other objects, advantages and features of the invention will become apparent to those persons skilled in the art upon reading the details of the compounds and methods more fully described below.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIG. 1 illustrates SEQ ID NO: 1, the polypeptide sequence of Beta-Amyloid (βA) as contained in Homo sapiens Beta-Amyloid Precursor Protein (APP_{770}) in GenBank Accession No. QRHUA4 from positions 672 to 714.

FIG. 2 illustrates SEQ ID NO: 2, the entire polypeptide sequence of APP_{770} in Homo sapiens.

FIG. 3 illustrates SEQ ID NO: 3, the polypeptide sequence of Prion Protein in Homo sapiens.

FIG. 4 illustrates SEQ ID NO: 4, the polypeptide sequence of α-Synuclein in Homo sapiens.

FIG. 5 illustrates SEQ ID NO: 5, the polypeptide sequence of Tau in Homo sapiens.

FIG. 6 illustrates SEQ ID NO: 6, the polypeptide sequence of Superoxide Dismutase in homo sapiens.

FIG. 7 illustrates SEQ ID NO: 7, the polypeptide sequence of Huntingtin in Homo sapiens.

FIG. 8 illustrates the first forty amino acids of βA_{1-40} incubated in the presence of Zn at a magnification of 1:52,000.

FIG. 9 illustrates the βA_{1-40} fibrils of FIG. 8 incubated with fusaric acid at a magnification of 1:52,000.

FIG. 10 illustrates the βA_{1-40} fibrils of FIG. 8 incubated with picolinic acid at a magnification of 1:52,000.

FIG. 11 illustrates βA_{1-40} fibrils in the presence of fusaric acid at a magnification of 1:52,000 becoming solubilized and forming fibril debris.

FIG. 12 illustrates βA_{1-40} fibrils in the presence of picolinic acid at a magnification of 1:52,000 becoming solubilized and forming fibril debris.

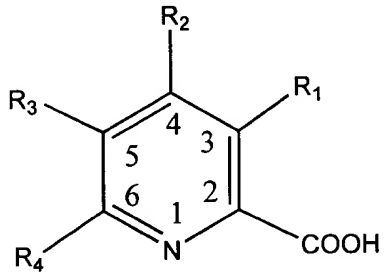
FIG. 13 illustrates a circular dichroism absorbance spectrum of a βA_{1-40} solution upon addition of Zn.

FIG. 14 illustrates a circular dichroism absorbance spectrum of a βA_{1-40} solution and the reduction of β-sheet conformation upon incubation with fusaric acid.

DETAILED DESCRIPTION OF THE INVENTION

It has been discovered that conditions, diseases and disorders related to prepathological and pathological protein assembly or aggregation caused by conformational changes of proteins from α-helical or random coil to β-sheet conformation may be treated with compositions that are capable of solubilizing conformationally altered proteins. The compositions of the invention, capable of solubilizing conformationally altered proteins, comprise a carboxylic acid anion of picolinic acid, its analogs, or derivatives and a cation. Picolinic acid, also known as α-pyridine carboxylic acid and 2-pyridine carboxylic acid, is a naturally occurring biological metabolite known to inhibit the growth of numerous cultured normal and transformed mammalian cells. Picolinic acid has the formula $C_6H_5NO_2$, a molecular weight of 123.11 g/mol and is readily soluble in water.

The compositions of the invention do not include zinc picolinate, chromium picolinate, molybdenum picolinate, iron picolinate, manganese picolinate, copper picolinate, boron picolinate or vanadium picolinate. In one embodiment of the invention, the carboxylic acid anion of picolinic acid, its derivatives, or analogs thereof is represented by the following structure:



wherein R_1, R_2, R_3 and R_4 are selected from the group consisting of an oligopeptide, carboxyl group, methyl group, ethyl group, propyl group, isopropyl group, butyl group, isobutyl group, secondary butyl group, tertiary butyl group, pentyl group, isopentyl group, neopentyl group, fluorine, chlorine, bromine, iodine and hydrogen.

In one embodiment, groups R_1, R_2 and R_4 are hydrogen and group R_3 is a butyl group. Fusaric acid, the 5-butyl derivative of picolinic acid, wherein R_1, R_2 and R_4 are hydrogen and R_3 is a butyl group, was first isolated from the fungus *Fusarium heterosporium* in 1934. Fusaric acid has the chemical name 5-butyl-2-pyndinecarboxylic acid, or 5-butylpicolinic acid, has a chemical formula of $C_{10}H_{13}NO_2$, a molecular weight of 179.22, and is readily soluble in water. The compositions described herein are readily commercially available, or may be made by methods well known in the art.

The oligopeptide may be in the range of about 10-24 amino acids, preferably in the range of about 14-20 amino acids, and more preferably, 16 amino acids. It will be appreciated that substitutions at the R_1, R_2, R_3 and R_4 positions are made with an oligopeptide having basic or acidic amino acids predominating. Such substituted analogs or derivatives have an increased molecular weight and a substantially increased half-life in the blood. Such compounds are also able to penetrate cells more effectively both *in vitro* and *in vivo* due to the amphipathic nature of the peptide residues. See, *e.g.*, U.S. Patent No. 6,127,393, hereby incorporated by reference in its entirety.

Pharmaceutically acceptable salts of picolinic acid and the above analogs may also be prepared from pharmaceutically acceptable non-toxic acids or bases including, but not limited to inorganic and organic acids. Buffering agents for picolinic acid or its analogs or derivatives may also comprise non-toxic acids or bases including, but not limited to inorganic or organic acids. Examples of such inorganic acids include, but are not limited to hydrochloric, hydrobromic, hydroiodic, sulfuric and phosphoric. Organic acids may be selected, for example, from aliphatic, aromatic, carboxylic and sulfonic classes of organic acids. Examples of suitable organic acids include, but are not limited to formic, acetic, propionic, succinic, glycolic, glucoronic, maleic, furoic, glutamic, benzoic, anthranilic, salicylic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, pantothenic, benzenesulfonic, stearic, sulfanilic, algenic and galacturonic acids. Examples of such inorganic bases for potential salt formation with the sulfate or phosphate compounds of the invention include, but are not limited to monovalent, divalent, or other metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc. Appropriate organic bases may also be selected from N,N-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumaine (N-methylglucamine),

procaine, ammonia, ethylenediamine, N-methyl-glutamine, lysine, arginine, omithine, choline, N,N'-dibenzylethylenediamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethylamine, diethylamine, piperazine, tris(hydroxymethyl)aminomethane and tetramethylammonium hydroxide.

The compositions of the invention also include a cation. Suitable cations include, but are not limited to aluminum, calcium, lithium, magnesium, potassium, sodium, ammonia, ethylenediamine, N-methyl-glutamine, lysine, arginine, omithine, choline, N, N'-dibenzyl ethylenediamine, chloroprocaine, diethanolamine, procaine, N-benzylphene-thymine, diethylamine, piperazine, tris(hydroxyethyl) aminomethane and tetramethylammonium hydroxide cations.

In one embodiment, the compositions of the invention include at least one buffering agent. Any buffering agent may be used. Suitable buffering agents include, but are not limited to hydrochloric, hydrobromic, hydroiodic, sulfuric, phosphoric, formic, acetic, propionic, succinic, glycolic, glucoronic, maleic, furoic, citric, glutamic, benzoic, anthranilic, salicylic, phenylacetic, mandelic, embonic, pamoic, methanesulfonic, ethanesulfonic, pantothenic, benzenesulfonic, stearic, sulfanilic, algenic, galacturonic acid and mixtures thereof. The buffering agents may comprise one or more additional agents including, but not limited to pregelatinized maize starch, polyvinyl pyrrolidone, hydroxypropyl methylcellulose, lactose, microcrystalline cellulose, calcium hydrogen phosphate, magnesium stearate, talc, silica, potato starch, sodium starch glycolate, sodium lauryl sulfate, sorbitol syrup, cellulose derivatives, hydrogenated edible fats, lecithin, acacia, almond oil, oily esters, ethyl alcohol, fractionated vegetable oils, methyl, propyl-p-hydroxybenzoates, sorbic acid and mixtures thereof. The buffering agents may also comprise at least one of dichlorodifluoromethane, trichloro fluoromethane, dichlorotetra fluoroethane, carbondioxide, poly (N-vinyl pyrrolidone), poly (methylmethacrylate), polyactide, polyglycolide and mixtures thereof.

In another embodiment, the buffering agent is formulated as at least one medium selected from a group, including, but not limited to a suspension, solution, or emulsion. In yet another embodiment, the buffering agent comprises a formulatory agent selected from the group including, but not limited to a carrier, excipient, suspending agent, stabilizing agent and dispersing agent.

It has been discovered that the above-described compositions of the invention may be used to successfully treat a wide range of conditions, diseases and disorders related to conformational changes of proteins from α-helical or random coil to β-sheet conformation. "Treatment" is preferably defined to cover any treatment of a disease in an animal, including a cow, sheep, deer, dog, cat, goat, chicken and turkey, particularly a human, and includes:

a) preventing the disease or symptom from occurring in a subject that may be predisposed to the disease or symptom but has not yet been diagnosed as having it;

b) inhibiting the disease or its symptom, *i.e.*, arresting development of the disease or symptom; or

c) relieving the disease or symptom, *i.e.*, causing regression or reversal of the disease or symptom.

The novel compositions of the invention disrupt the abnormal metal ligand/binding site complex of many proteins that cause degenerative diseases so that the protein will remain in its normal soluble state, or solubilize an already assembled or aggregated conformationally altered protein. Amino acid sequences of proteins that cause degenerative diseases are include: βA (FIG. 1; SEQ ID NO: 1), APP (FIG. 2; SEQ ID NO: 2), PrP (FIG. 3; SEQ ID NO: 3), α-Synuclein (FIG. 4; SEQ ID NO: 4), Tau (FIG. 5; SEQ ID NO: 5), Superoxide Dismutase (FIG. 6; SEQ ID NO: 6), and ~~Huntingtin~~ Huntington (FIG. 7; SEQ ID NO: 7). Accordingly, the compositions of the invention are used for treating diseases, disorders and conditions manifested by prepathological and pathological protein assembly or aggregation wherein the assembled or aggregated protein contains at least one protein selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7.

FIG. 1 shows SEQ ID NO: 1, the polypeptide sequence of Beta-Amyloid (βA) as contained in Homo sapiens Beta-Amyloid Precursor Protein (APP_{770}) in GenBank Accession No. QRHUA4 from positions 672 to 714 ~~available at http://www.nebi.nlm.nih.gov; herein incorporated by reference in its entirety~~. The term "β-amyloid," "β-amyloid peptide" or "βA" refers to a 39-43 amino acid peptide having a molecular weight of about 4.2 kDa, which peptide is substantially homologous to the form of the protein described by Glenner, *et al.* including mutations and post-translational modifications of the normal β-amyloid peptide (Glenner et al., *Biochem. Biophys. Res. Commun.* 120:885-890 (1984)), and comprising SEQ ID NO: 1, as well

as biologically active variants of SEQ ID NO: 1, which has at least about 80%, preferably at least about 90%, and more preferably at least about 95%, identity or homology to SEQ ID NO: 1 or a biologically active subunit thereof. Biologically active subunits of βA, biologically active variant βA polypeptides, and biologically active subunits thereof, falling within the scope of the invention, have at least about 50%, preferably at least about 80%, and more preferably at least about 90% the activity of the polypeptide comprising SEQ ID NO: 1.

FIG. 2 shows SEQ ID NO: 2, the entire polypeptide sequence of APP_{770} in Homo sapiens as listed in GenBank Accession No. QRHUA4. The term "β-Amyloid Precursor Protein" or "APP" refers to a 770 amino acid peptide having a molecular weight of about 87.0 kDa, comprising SEQ ID NO: 2 which has at least about 80%, preferably at least about 90%, and more preferably at least about 95%, identity or homology to SEQ ID NO: 2 or a biologically active subunit thereof. Biologically active subunits of APP, biologically active variant APP polypeptides, and biologically active subunits thereof, falling within the scope of the invention, have at least about 50%, preferably at least about 80%, and more preferably at least about 90% the activity of the polypeptide comprising SEQ ID NO: 2.

FIG. 3 shows SEQ ID NO: 3, the polypeptide sequence of Prion Protein in Homo sapiens as listed in GenBank Accession No. XM009567. The term "Prion Protein" or "PrP" refers to a 253 amino acid peptide having a molecular weight of about 27.7 kDa, comprising SEQ ID NO: 3 which has at least about 80%, preferably at least about 90%, and more preferably at least about 95%, identity or homology to SEQ ID NO: 3 or a biologically active subunit thereof. Biologically active subunits of PrP, biologically active variant PrP polypeptides, and biologically active subunits thereof, falling within the scope of the invention, have at least about 50%, preferably at least about 80%, and more preferably at least about 90% the activity of the polypeptide comprising SEQ ID NO: 3.

FIG. 4 shows SEQ ID NO: 4, the polypeptide sequence of α-Synuclein in Homo sapiens as listed in GenBank Accession No. XM003494. The term "α-synuclein" refers to a 140 amino acid peptide having a molecular weight of about 14.5 kDa, comprising SEQ ID NO: 4 which has at least about 80%, preferably at least about 90%, and more preferably at least about 95%, identity or homology to SEQ ID NO: 4 or a biologically active subunit thereof. Biologically active subunits of α-synuclein, biologically active variant α-synuclein polypeptides, and

biologically active subunits thereof, falling within the scope of the invention, have at least about 50%, preferably at least about 80%, and more preferably at least about 90% the activity of the polypeptide comprising SEQ ID NO: 4.

FIG. 5 shows SEQ ID NO: 5, the polypeptide sequence of Tau in Homo sapiens as listed in GenBank Accession No. NM016835. The term "Tau" refers to a 758 amino acid peptide having a molecular weight of about 78.9 kDa, comprising SEQ ID NO: 5 which has at least about 80%, preferably at least about 90%, and more preferably at least about 95%, identity or homology to SEQ ID NO: 5 or a biologically active subunit thereof. Biologically active subunits of Tau, biologically active variant Tau polypeptides, and biologically active subunits thereof, falling within the scope of the invention, have at least about 50%, preferably at least about 80%, and more preferably at least about 90% the activity of the polypeptide comprising SEQ ID NO: 5. ~~In particular, the biologically active variants of Tau can include peptides listed in GenBank at Accession Nos. NM_016841, NM_016834, and NM_005910.~~

FIG. 6 shows SEQ ID NO: 6, the polypeptide sequence of Superoxide Dismutase in homo sapiens as listed in GenBank Accession No. P00441. The term "superoxide dismutase" refers to a 154 amino acid peptide having a molecular weight of about 15.9 kDa, comprising SEQ ID NO: 6 which has at least about 80%, preferably at least about 90%, and more preferably at least about 95%, identity or homology to SEQ ID NO: 6 or a biologically active subunit thereof. Biologically active subunits of superoxide dismutase, biologically active variant superoxide dismutase polypeptides and biologically active subunits thereof, falling within the scope of the invention, have at least about 50%, preferably at least about 80%, and more preferably at least about 90% the activity of the polypeptide comprising SEQ ID NO: 6. In particular, the biologically active variants of superoxide dismutase can include the Cu-Zn family of superoxide dismutases as well as the Mn family of superoxide dismutases.

FIG. 7 shows SEQ ID NO: 7, the polypeptide sequence of Huntingtin in Homo sapiens as listed in GenBank Accession No. XP003405. The term "Huntingtin" refers to a 1543 amino acid peptide having a molecular weight of about 168.8 kDa, comprising SEQ ID NO: 7, which has at least about 80%, preferably at least about 90%, and more preferably at least about 95% identity or homology to SEQ ID NO: 7, or a biologically active subunit thereof. Biologically active subunits of Huntingtin, biologically active variant Huntingtin polypeptides, and biologically

active subunits thereof, falling within the scope of the invention, have at least about 50%, preferably at least about 80%, and more preferably at least about 90% the activity of the polypeptide comprising SEQ ID NO: 7.

In accordance with the present invention, diseases caused by biologically active subunits of these proteins may also be treated by administration of the compounds of the invention. The term "biologically active subunit" of a peptide is preferably defined to mean a subunit of a peptide of the invention, including SEQ ID NO: 1 through SEQ ID NO: 7, which has at least about 10%, preferably at least about 50%, and more preferably at least about 90% activity of a peptide of the invention. A biologically active subunit will have at least one metal-binding site. The activity of a peptide of the invention can be measured by methods well known in the art including, but not limited to the ability of the peptide to bind a transition metal ion *in vitro*, or the ability of the peptide to change conformation from α-helical or random coil to β-sheet conformation as visualized by changes in optical rotary dispersion (ORD) or circular dichroism (CD), and comparing these results to results obtained from wild-type peptide activity. Accordingly, and in one embodiment of the invention, the above-described compositions are used for treating diseases manifested by prepathological and pathological protein assembly or aggregation of a biologically active subunit of a protein selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7.

The term "metal-binding site" is preferably defined to mean a three-dimensional structure comprising at least one amino acid exhibiting steric complementarity between it and a metal ligand, preferably a transition metal ion. Two or more amino acids may form the three-dimensional structure either sequentially connected within a single polypeptide, from different sites within a single polypeptide, or from different polypeptides, exhibiting steric complementarity between it and a metal ligand, preferably a transition metal ion. It is well known in the art that metal ions tend to bind to protein groups for which they have some intrinsic affinity. Thus, both characterized and uncharacterized metal binding sites of SEQ ID NO: 1 through SEQ ID NO: 7 that can be predicted by methods well known in the art, *e.g.*, by using computer software techniques or chemosensors, are within the scope of the invention.

For example, it is well known in the art that Zn(II) ions tend to be bound to sulfur atoms and to the imidazole nitrogen atoms of *His* residues; Fe(II) and Fe(III) to the sulfur atoms of *Cys* residues or to sulfide ions and in iron-sulfur proteins; Ca(II) ions tend to interact with thiol or imidazole groups; Ca(II) ions tend to be bound to oxygen atoms; and, Mg(II) ions are bound along with the phosphate groups of ligands. In addition, metal-binding site sequences, including but not limited to Zn-finger, Fe-finger, and Fe-ring structures, may be predicted by chemosensors such as those described in U.S. Patent No. 6,083,758 to Imperiali, *et al.*, herein incorporated by reference in its entirety.

Computer software using predictive algorithms may also be used to determine the existence of a metal-binding site. Data representing the intrinsic metal-binding affinities of certain amino acids in cooperative conformations can be used by the algorithm to predict the amino acids comprising a metal-binding site and the affinity for metal ligands.

The activity of a metal-binding site of the invention can be measured by methods well known in the art including, but not limited to the ability of the peptide to bind a transition metal ion *in vitro*, or the ability of the peptide to change conformation from α-helical or random coil to β-sheet conformation as visualized by changes in ORD or CD, and comparing these results to results obtained from wild-type peptide activity.

The metal-binding sites of these proteins will be present in certain biologically active subunits and/or biologically active variants of the proteins of FIG. 1 through FIG. 7 (SEQ ID NO: 1 through SEQ ID NO: 7). The use of the compositions of the invention to treat diseases manifested by the assembly or aggregation of these biologically active subunits and/or biologically active variants is intended to fall within the scope of the invention. Therefore, the protein targets of the invention include proteins having at least about 80%, preferably at least 90%, and more preferably at least about 95% identity to the above disclosed proteins of FIG. 1 through FIG. 7 (SEQ ID NO: 1 through SEQ ID NO: 7).

The following Table is a non-exclusive list of diseases, disorders and conditions, and their associated proteins, that assume two or more different protein conformations, one of which causes a manifestation of the disease.

Disease		Insoluble Proteins
Alzheimer's Disease		APP, βA, α1-antichymotrypsin, tau, non- βA component
Prion Diseases, Creutzfelds-Jakob disease, scrapie and bovine spongiform encephalopathy		PrP^{Sc}
ALS		SOD and neurofilament
Pick's Disease		Pick body
Parkinson's Disease		α-synuclein in Lewy bodies
Frontotemporal dementia		Tau in neurofibrillary tangles
Type II Diabetes		Amylin
Multiple myeloma-plasma cell dyscrasias		IgG L-chain
Familial amyloidotic polyneuropathy		Transthyretin
Medullary carcinoma of thyroid		Procalcitonin
Chronic renal failure		B_2-microglobulin
Congestive heart failure		Atrial natriuretic factor
Senile cardiac and systemic amyloidosis		Transthyretin
Chronic inflammation		Serum amyloid A
Atherosclerosis		ApoA1
Familial amyloidosis		Gelsolin
Huntington's Disease		Huntingtin

In accordance with the present invention, diseases caused by biologically active variants of the above proteins are also treated by the introduction of the composition of the invention to the variants. The term "biologically active variant" of a protein is preferably defined to mean a protein, which has at least about 80%, preferably at least about 90%, more preferably 95% identity or homology to a protein of the invention, including SEQ ID NO: 1 through SEQ ID NO: 7. Biologically active variants of the peptides of the invention have at least about 10%, preferably at least 50%, and more preferably at least about 90% activity of a protein of the invention. A biologically active subunit will have at least one metal-binding site. The activity of a variant peptide or protein of the invention can be measured by methods well known in the art including, but not limited to the ability of the peptide to bind a transition metal ion *in vitro*, or the

ability of the peptide to change conformation from α-helical or random coil to β-sheet conformation as visualized by changes in ORD or CD, and comparing these results to results obtained from wild-type peptide activity. Therefore, the compounds of the invention may be used for treating diseases manifested by prepathological and pathological protein assembly or aggregation of a biologically active variant of a protein selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7.

Many of the above proteins are metalloproteins that play a role similar to metalloproteins in cancers, pain, inflammation, proliferative and infectious diseases. It is well known that metalloproteins that change conformation by binding a transition metal ion can activate certain enzymes, viruses and cancers. For example, metal-binding site motifs, such as zinc-finger or zinc-ring sequences, play an important role as hormone-receptor proteins and in proliferative, inflammatory and infectious diseases. Inhibition of zinc-finger proteins results in potent anti-cancer and anti-viral effects *in vivo*. The effect of picolinic acid and fusaric acid, and their analogs or derivatives, on cancers and viruses is detailed in U.S. Patent Nos. 6,127,393 and 5,767,135, herein incorporated by reference in their entireties.

Proteins having metal-binding sites, particularly those sites complementary to transition metal ions, appear to be effective targets for treatment of diseases caused by conformational alteration of these proteins. Different conformations of the same proteins, having the same primary amino acid sequence, can have dramatically different activities *in vivo*. For example, AD and PrP infection are believed to be caused by transition metal ion related conformation changes from normally soluble α-helical or random coil to insoluble β-sheet structures. Accordingly, disrupting the metal binding and β-sheet formation of the proteins underlying these diseases is an effective mode of treating the diseases.

Zn-, Cu- and/or Fe-binding sites on βA and PrP are required for the assembly and aggregation of proteins causing Alzheimer's Disease, FAD, CAA, spongiform encephalopathies and prion diseases which include kuru, CJD, Gerstmann-Sträussler-Sheinker Disease (GSS), fatal familial insomnia (FFI) and related scrapie diseases. It is also believed that metal-binding sites exist within other proteins manifesting in diseases related to the prepathological and pathological assembly or aggregation of the proteins. Such pathologies include: Parkinson's

disease, frontal temporal dementia, Pick's disease, amyotrophic lateral sclerosis and Huntington's disease. Other diseases manifested by the assembly or aggregation of conformationally altered proteins, including the proteins of FIGS. 1 through FIG. 7 (SEQ ID NO: 1 through SEQ ID NO: 7), requiring Zn, Cu and/or Fe to assemble and/or aggregate can be successfully treated by the introduction of the compositions of the invention to those conformationally altered proteins. Thus, an effective treatment of these diseases, involving abnormal metal-binding and prepathological and pathological protein assembly or aggregation, is administering the compositions of the invention to disrupt, or interfere with, the formation or action of these metal-binding proteins to prevent, inhibit, stop, and/or reverse the progress of the related diseases. Accordingly, and in one embodiment of the invention, conformationally altered protein assembly or aggregation in an animal is reversed or prevented by introducing the above-described compositions of the invention to the conformationally altered protein. In a specific embodiment, picolinic acid, its analogs, or derivatives is introduced to the conformationally altered protein. In another specific embodiment, fusaric acid is introduced to the conformationally altered protein.

In one embodiment of the invention, Alzheimer's Disease is treated by the introduction or administration of the compositions of the invention to the affected animal. AD is a progressive neurodegenerative disorder characterized by extracellular deposits of βA, the main component of neuritic or senile and diffuse plaques. The βA_{1-40} isoform is the predominant soluble species in biological fluids. Although less abundant in biological fluids, βA_{1-42} is found in higher concentrations in plaque deposits.

Pathogenic mutations of the APP gene close to or within the βA domain are linked to forms of FAD. Inheritance of mutations on chromosome 14 (presenilin-1), or chromosome 1 (presenilin-2) produces the more aggressive form of the disease manifesting in early-onset at age 25 to 45 years. Under normal physiological conditions, βA is a soluble cellular metabolite that is produced by a variety of cells and is found in the cerebrospinal fluid and plasma. However, βA in the neuritic or senile and diffuse plaques is in the form of amyloid fibrils that are insoluble under physiological conditions. It is likely that neurochemical factors, having levels altered through the course of aging, initiate βA deposition in sporadic AD and FAD. Thus, the plaque deposits of βA appear to be a morphological variation of βA accumulation caused by

neurochemical interactions that are specific to the neocortex. The availability of high concentrations of Cu(II) and Zn(II) is a specific feature of neocortical tissue that explains the condensation of βA as plaque.

βA deposition in the neocortex is closely related to the pathology of AD. The deposition of βA in the neocortex of APP transgenic mice overexpressing βA is accompanied by some neuropathological features of AD such as neuronal loss suggesting that the neurotoxic events of AD are related to βA accumulation. In addition, many studies have now confirmed that βA is neurotoxic in cell culture and *in vivo*.

A consensus has emerged among researchers that the homeostases of Zn(II), Cu(II), and Fe(III) are significantly altered in AD brain tissue. For example, abnormal levels of Zn(II), Cu(II), or Fe(III) have been found in several subcortical regions such as the hippocampus, amygdala, and olfactory bulb, as well as the neocortex.

Synthetic βA and purified APP exhibit several physicochemical interactions with Zn(II), Cu(II), and to a lesser extent Fe(III), at low micromolar and submicromolar concentrations of the metal ions. Although the transition metal ions Zn, Cu, and Fe are maintained at high concentrations within the healthy brain neocortical parenchyma, increased concentrations of these metal ions are detected in the neuropil of the AD-affected brain, where they are highly concentrated within amyloid plaque deposits. An elevated Zn(II) concentration can also be detected in plaque deposits histologically. βA has also been found to avidly bind Zn(II), Cu(II), and Fe(III) *in vitro*, suggesting that these metals are an important factor in amyloid plaque pathology.

The roles these metal ions play in cerebral amyloid assembly and aggregation have been further characterized by experiments showing that Zn(II)- and Cu(II)-selective chelators enhance the solubilization of βA collections in postmortem brain specimens from AD subjects and from amyloid precursor protein transgenic mice. Specific and saturable Zn-binding sites have been identified at positions 181-200 and 135-155 of APP_{695} ($APP_{181-200}$ and $APP_{135-155}$) of FIG. 2 (SEQ ID NO: 2). Additional Zn- and Cu-binding sites have been found at positions 108, 110, 147, 149, 151, 183, 186, and 187 of APP_{770}. FIG. 2 (SEQ ID NO: 2) below is the entire 770 amino acid sequence of APP_{770} that contains the APP_{695} splice form by connection of positions 1-288 and 365-770. These sites have homology in all known members of the APP superfamily.

This indicates that Zn and Cu interaction with the protein may play an important, evolutionary conserved role in APP function and metabolism.

βA_{1-40} specifically and saturably binds Zn(II), Cu(II) and Fe(III). The *His* residue, at position 13 of FIG. 1 (SEQ ID NO: 1), is believed to be an important residue in Zn-mediated βA assembly. The *His* residue, at position 14 of FIG. 1 (SEQ ID NO: 1), is also an important residue in Zn-mediated βA assembly. The entire Zn-binding site between positions 6 and 28 of FIG. 1 (SEQ ID NO: 1) has been characterized.

Further three dimensional analysis of βA residues 11-25, believed to be the core domain of the βA fibril, have also been resolved, suggesting that βA in plaques take a β-hairpin conformation in one species of the β-sheet conformation in AD. It is believed that multiple β-hairpin fragments further form a protofilament, or stacking of βA β-hairpin units, and later assemble to form the neurotoxic plaque with five or six protofilaments forming a hollow tube, or β-sheet crystallite. Both βA and a fragment of the Prion Protein (PrP) exist in a pentagonal or hexagonal array of β-sheet crystallites.

The βA_{1-42} isoform was discovered to initiate aggregation and assembly of βA_{1-40}. It was also determined that the addition of Zn(II), Cu(II), and Fe(III) both enhance and are required for the βA_{1-42}-initiated seeding of βA_{1-40}. Both Zn- and Cu-induced aggregation and assembly of βA_{1-40} is reversible by chelation with strong Zn/Cu chelators such as N,N,N',N'-tetrakis-(2-pyridylmethyl)-ethylenediamine and bathocuproine disulfonic acid *in vitro*. Thus, solubilization of βA plaque may be accomplished by chelating the Zn, Cu, and Fe bound to βA. Such Zn/Cu chelators, however, exhibit toxic effects *in vivo* and are not believed to be able to pass the blood-brain barrier upon administration by inhalation, insufflation, oral, buccal, parenteral, transdermal, or rectal administration.

The PrP gene in mammals expresses a protein that can be either a soluble, non-disease form (PrP^C), or in an insoluble, disease causing form (PrP^{Sc}). PrP^C is encoded by a single-copy host gene and is generally found on the outer surface of neurons. FIG. 3 (SEQ ID NO: 3) shows the translated product of the single-copy gene in Homo sapiens. It is thought that prion diseases result from the transformation of PrP^C into PrP^{Sc} by changing from an α-helical conformation to a β-sheet conformation. Currently, the only disease-specific diagnostic marker of prion diseases is the presence of PrP^{Sc} in the brains of infected animals, including humans. Although there is no

difference in the primary amino acid sequence of the two forms, PrP^{Sc} has a conformation with higher β-sheet and lower α-helix or random coil content than PrP^{C}. Transition metal ions are believed to be required for the conformation change.

PrP^{Sc} plays a key role in both transmission and pathogenesis of prion diseases (*e.g.*, spongiform encephalopathies) and it is a critical factor in neuronal degeneration. Four prion diseases of humans have been identified: kuru, CJD, GSS, and FFI. The most common prion diseases in animals are scrapie in sheep and goats and bovine spongiform encephalopathy (BSE) in cattle. These diseases are thought to be able to spread across species to other hoofed animals, such as deer, and potentially other staple animals such as chickens, turkeys, dogs and cats. At the time of the invention, scrapie and related diseases have caused billions of dollars in damage related to enacting quarantine procedures and destroying livestock suspected of being infected with PrP. There is currently no treatment for these diseases.

It will be appreciated by those skilled in the art that other diseases, including, but not limited to Parkinson's disease, frontal temporal dementia, Pick's disease, amyotrophic lateral sclerosis, and Huntington's disease, that are caused by assembly or aggregation of conformationally altered proteins, and that comprise SEQ ID NO: 4 through SEQ ID NO: 7 (FIG. 4 through FIG. 7) contain at least one metal-binding motif that can be predicted by methods well known in the art, *e.g.*, by using computer software techniques or chemosensors. The treatment of the aforementioned diseases manifested by assembly or aggregation of the above-described proteins, biologically active subunits and biologically active variants thereof by the introduction of the compositions of the invention to the conformationally altered protein is intended to be within the scope of the invention.

In vitro studies reveal that picolinic acid and fusaric acid have activity in cell-free systems that allow examination of the formation and reversal of formation of βA fibrillar deposits. CD spectroscopy and electron microscopy have previously shown that Zn is required for conformational changes of βA.

CD spectroscopy is based on the principle that the L-amino acids in polypeptides and proteins interact differently with beams of left-and right-circularly polarized light, which causes the beams to travel at different speeds through these molecules, thereby rotating the polarized light. Left- and right-circularly polarized beams of light are also absorbed to different extents by

chiral molecules. Relative amounts of random coiled, α-helix and β-sheet conformations are readily resolved by comparing CD absorption bands to control data. For example, the absorption spectra for an oligopeptide such as poly(*Lys*) show distinctive absorption spectra for the random coil, α-helix and β-sheet conformations of the polypeptide:

Random coil:	Minima of about –40 degree cm^2/decimole at about 197 nm. Maxima of about 5 degree cm^2/decimole at about 218 nm.
α-helix:	Minima of about –32 and –35 degree cm^2/decimole at about 208 and 222 nm respectively. Maxima of about 78 degree cm^2/decimole at about 192 nm.
β-sheet:	Minima of about –18 degree cm^2/decimole at about 218 nm. Maxima of about 32 degree cm^2/decimole at about 195 nm.

The compositions of the invention described herein are administered to a patient at a therapeutically effective dose to treat or ameliorate neurodegenerative disorders, *e.g.*, amyloidoses, prion diseases, and other degenerative diseases. The term "therapeutically effective" dose is preferably defined to mean an administration of the compounds of the invention sufficient to provide the desired physiological and/or psychological change. This will vary depending on the patient, the disease and the treatment. The dose may either be a therapeutic dose, in which case it sufficiently alters levels of conformationally altered protein deposits in the subject to alleviate or ameliorate the symptoms of the disorder or condition, or a prophylactic dose, which is be sufficient to prevent accumulation of insoluble protein deposits to an undesired level.

Toxicity and therapeutic efficacy of the compounds of the invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such compounds to the

site of affected tissue in order to minimize potential damage to unaffected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

The dosage also depends on the patient and condition being treated and on the administration route. For example, the dosage depends upon the efficacy of a therapeutic effect for different mammals, often requiring widely varying doses, and the mode of administration, *e.g.*, oral doses may often be ten times the injection dose because of the degradation of a compound that may occur in the stomach.

Pharmaceutical compositions for use in the invention can be formulated in a conventional manner using one or more pharmaceutically acceptable carriers or excipients. Thus, the compositions of the invention can be formulated for administration by inhalation, insufflation (either through the mouth or the nose), oral, buccal, parenteral, transdermal, or rectal administration.

For oral administration, the pharmaceutical compositions can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients including, but not limited to binding agents, *e.g.*, pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose; fillers, *e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate; lubricants, *e.g.*, magnesium stearate, talc or silica; disintegrants, *e.g.*, potato starch or sodium starch glycolate; and, wetting agents, *e.g.*, sodium lauryl sulfate. The tablets can be coated by methods well known in the art. Liquid preparations

for oral administration can take the form of, for example, solutions, syrups or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives including, but not limited to suspending agents, *e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats; emulsifying agents, *e.g.*, lecithin or acacia; non-aqueous vehicles, *e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils; and preservatives, *e.g.*, methyl or propyl-*p*-hydroxybenzoates or sorbic acid. The preparations can also contain buffering agents, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration can be suitably formulated to give controlled release of the active compound, *e.g.*, time release formulations for predetermined dosage release over predetermined time periods. For buccal administration, the compositions can take the form of tablets or lozenges formulated in a conventional manner.

For administration by inhalation, the compositions of the invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a neubulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator, can be formulated containing a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

The compositions of the invention can be formulated for parenteral administration (*i.e.*, intravenous or intramuscular) by injection, via, for example, bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compositions of the invention can be formulated for transdermal administration in the form of permeable membranes placed directly on the stratum corneum (*i.e.*, the outer most

layer of skin). Formulations for transdermal administration can include suitable carriers, *e.g.*, poly(N-vinyl pyrrolidone), poly(methyl methacrylate), polylactides, and polyglycolides. The permeation of drugs through skin can also be enhanced by physical methods such as iontophoresis (*i.e.*, application of low level electric current), phonophoresis (*i.e.*, use of ultra sound energy) and by chemical penetration enhancers, *e.g.*, fatty acids, fatty alcohols and terpenes.

The compositions of the invention can also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides. In addition to the formulations described previously, the compositions can also be formulated as depot preparations. Such long acting formulations can be administered by implantation, for example, subcutaneously or intramuscularly, or by intramuscular injection. Thus, for example, the compounds can be formulated with suitable polymeric or hydrophobic materials, *e.g.*, as an emulsion in an acceptable oil, or ion exchange resins, or as sparingly soluble derivatives, for example as a sparingly soluble salt.

The compositions of the invention can, if desired, be administered in a pack or dispenser device that can contain one or more unit dosage forms containing the active ingredient. The pack can for example comprise metal or plastic foil, such as a blister pack. In addition, the compositions may be administered in an ampoule or multi-dose container. The pack or dispenser device can be accompanied by instructions for administration.

The following examples are illustrative of specific embodiments of the invention and do not limit the scope of the invention in any way.

EXAMPLE 1- Zn-MEDIATED βA POLYMER FORMATION AND ITS REVERSAL BY PICOLINIC OR FUSARIC ACID

Negative stain electron microscopy (EM) reveals Zn-induced polymerization and fibril formation of βA_{1-40} and the effect of fusaric acid and picolinic acid as seen in FIGS. 8 through 14. βA_{1-40} may be prepared by *in vitro* methods well known in the art, such as solid phase protein synthesis or t-Boc and CBZ protein synthesis. *See* Tarbell, D.S., Yamamoto, Y. & Pope, B.M. *Proc. Natl. Acad. Sci. USA* 69, 730-732 (1972), herein incorporated by reference in its entirety. The peptide was purified by C18 reverse phase HPLC and lypholized. Lypholized

peptides were then dissolved in aqueous buffers such as phosphate buffered saline (PBS). High purity of the peptides was determined by mass spectrometry and quantitative amino acid analysis.

When βA_{1-40} was incubated in PBS at 37°C in the presence of 10 μM Zn acetate at pH 6.5, it formed lateral polymers and fibril aggregates. FIG. 8 shows βA_{1-40} incubated in the presence of Zn at a magnification of 1:52,000. These lateral fibrils are formed *de novo* upon adding Zn to the solution containing soluble βA_{1-40}. These lateral fibril aggregates are reversible and require the presence of Zn for formation. Peptides were then diluted to a final concentration of 300 μM in aqueous buffers with the Zn concentration adjusted to 3 mM for 2 hours. For negative staining, 10 μl of each sample was applied to Pioloform® (Wacker Polymer Systems, GmbH, Germany) and carbon-coated grids, blotted with filter paper and stained with 1% (w/v) phosphotungstic acid having a pH of 7.0. The specimens were examined on a Hitachi H7000 electron microscope (Hitachi Corp., Japan) with an accelerating voltage of 75 kV.

Polymerization and fibril formation was prevented by incubation of the mixture under identical conditions in the presence of 100 μM picolinic or fusaric acid. FIG. 9 shows the βA_{1-40} fibrils of FIG. 8 incubated with fusaric acid at a magnification of 1:52,000. No additional Zn-mediated polymerization of βA_{1-40} fibrils was observed upon incubation with fusaric acid. FIG. 10 shows the βA_{1-40} fibrils of FIG. 8 incubated with picolinic acid at a magnification of 1:52,000. No additional Zn-mediated polymerization of βA_{1-40} fibrils was observed upon incubation with picolinic acid. In addition, when preformed fibrils (formed in the presence of Zn) were incubated with picolinic or fusaric acid, the fibril aggregates dissociated. FIG. 11 shows the βA_{1-40} fibrils in the presence of fusaric acid at a magnification of 1:52,0000 becoming solubilized and forming fibril debris. FIG. 12 shows the βA_{1-40} fibrils in the presence of picolinic acid at a magnification of 1:52,0000 becoming solubilized and forming fibril debris. This indicates that both picolinic and fusaric acid are able to prevent the formation of conformationally altered βA_{1-40} polymerization, which requires zinc binding and to disassociation of bound Zn, thereby causing conformational changes in βA_{1-40} and resulting in reversal of βA_{1-40} polymerization.

EXAMPLE 2 - REVERSAL OF βA AGGREGATION BY CHANGING PROTEIN CONFORMATION

CD spectroscopy demonstrates that picolinic acid and fusaric acid prevent and reverse βA_{1-40} fibril formation by changing the conformational structure of the protein. βA_{1-40} was solubilized in filtered 20 mM sodium phosphate buffer having a pH of 7.0 to a final peptide concentration of 55 μM. Zn(II) was added to βA_{1-40} in 20 mM Tris buffer having a pH of 7.0 at a molar ratio of 1:10. The samples were allowed to stand for 10 minutes at room temperature prior to analysis. CD spectra were then acquired on a Jasco spectropolarimeter Model J-715 (Jasco Corp., Japan) at room temperature in a 0.1-cm path length cell over the wavelength range 190-250 nm with a 1.0 nm bandwidth, 0.1 nm resolution, 1 second response time and 20 nm/minute scan rate.

The β-sheet conformation is demonstrated by the presence of absorbance maxima at 202 nm. FIG. 13 shows a CD absorbance spectrum of a βA_{1-40} solution upon addition of Zn. The x-axis is measured in nm and the y-axis is measured in degree cm^2/decimole. The formation of the βA_{1-40} β-sheet conformation is confirmed by observing the formation of a peak at approximately 202 nm. However, in the presence of a small molar excess of picolinic acid or fusaric acid, the Zn-dependent conversion of the βA_{1-40} from the random coil to the β-sheet conformation is prevented as seen by the decrease of the 202 nm peak. FIG. 14 shows a CD absorbance spectrum of a βA_{1-40} solution and the reduction of the β-sheet conformation upon incubation with fusaric acid. The reduction can be seen by observing the decreasing peak at approximately 202 nm from hour 0, to hour 1, to hour 24. Once the β-sheets of βA_{1-40} are formed in the presence of Zn, they are reversed by the addition of either picolinic acid or fusaric acid. The loss of β-sheet conformation is due to Zn sequestration by picolinic acid or fusaric acid. Thus, the reversal of protein aggregation by picolinic acid and fusaric acid is directly linked to the ability to alter protein conformation by disrupting, or interfering with, Zn binding to the Zn-binding site. This change in protein conformation results in the prevention and reversal of protein fibril formation and solubilization of fibrils and aggregates.

EXAMPLE 3 - RELEASE OF βA FROM HUMAN BRAIN TISSUE

A series of picolinic acid and fusaric acid incubations show that picolinic and fusaric acid release βA directly from brain slices from post mortem AD patients. Small amounts of brain tissue were bathed in a predetermined volume of physiological buffer solution containing either 1mM picolinic acid or fusaric acid for 8-12 hours at 4°C with gentle mixing. At the end of the incubation, the amount of amyloid protein released from the tissue was quantitated using Enzyme-Linked Immunosorbent Assay (ELISA) techniques well known in the art and a monoclonal antibody specific for βA_{1-40}.

Human brain tissue from deceased Alzheimer's dementia patients was used to determine the activity of picolinic and fusaric acids in the release of βA from neurofibrillar plaques present in abundance in this tissue. This study was performed to demonstrate the reversal of full-length plaque protein due to βA deposition formed *in vivo*. After incubation under physiological conditions, the amount of specific βA solubilized and released from the brain tissue was quantitated using an immunoassay with an antibody specific the βA. Data from a representative *ex vivo* study is shown in TABLE 2. The data indicate that picolinic acid and fusaric acid mediate the release of full length βA formed *in vivo* from human brain tissue slices.

TABLE 2 - Release of β-Amyloid Protein from Human Brain Tissue by Various Agents

Condition	Patient AD2 set 1 (cts $x10^{-3}$)	Patient AD2 set 2 (cts $x\ 10^{-3}$)
Blank	313	358
Fusaric acid, 1mM	450	449
Picolinic Acid, 1mM	422	416
EDTA, 1mM	256	325

This representative data indicates that both fusaric acid and picolinic acid promote the release of βA from plaques in human brain tissue that is greater than that released by the well-characterized chelator EDTA.

Although preferred embodiments of the invention have been described in the foregoing Detailed Description of the Invention, it will be understood that the invention is not limited to the

embodiments disclosed but is capable of numerous modifications without departing from the spirit and scope of the present invention.